

## TRACK Number

# **Brushing Your Spacecraft's Teeth:** ***A Review of Bioburden Reduction Processes*** ***for Planetary Protection Missions***

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# Outline

- Introduction: Why Brush Your Spacecraft's Teeth?
- Brush and Swish
- Deep Cleaning
- The Whole Mouth
- Flossing In-Between
- Fresh Breath and Clean Teeth

# Outline

## What this is:

- An orientation guide to the world of biology for planetary protection missions
- A different way to think about the materials selection process for planetary science missions.

## What this isn't:

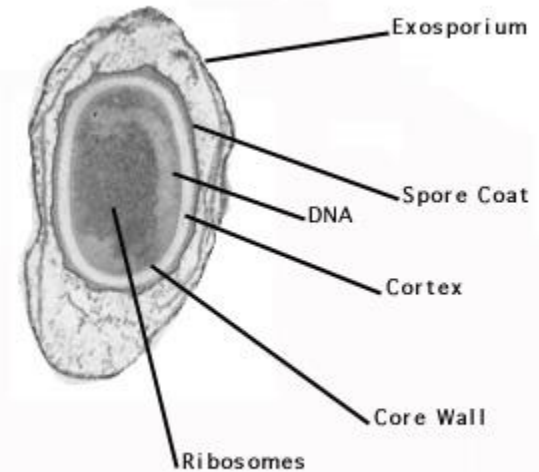
- A review of planetary protection policy
- A Headquarters-directed list of what can or cannot/should or should not be used.

# Introduction

Planetary Protection: NASA planetary science missions are required to comply with requirements that protect the science and protect the planet, depending on the mission's objectives.

# Why Brush Your Spacecraft's Teeth?

Most cleanrooms exist at 70F/20C and 50% humidity.



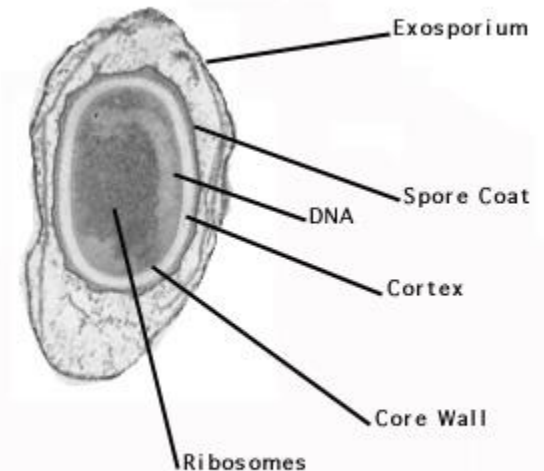
# Why Brush Your Spacecraft's Teeth?

Take a census by  
counting.

Counting What?  
Counting Spores.

Bacteria can form a metabolically inactive state when environmental conditions are harsh.

When we take a census, we heat shock the collected bacteria to drive spore formation.-- looking for the hardiest critters, those that could potentially survive the trip to another place in our solar system.



# Log Reduction

“Log Reduction” is not about chopping firewood...

**START** With a known level of spores  
*You know this level because you’ve sampled and assayed.*

**SELECT** A bioburden process that has been well characterized (e.g. NASA Standard Process)

**APPLY** that process to your hardware with appropriate level of controls.

**END RESULT:** a number of logs of reduction in spores

# Methods of Bioburden Reduction

**Methods of bioburden reduction fall into two main camps:**

- **Surface**
- **Penetrating**

## Surface

- Addresses exterior portions of hardware only
- Can include: piece parts, components prior to integration

**Common Methods:**

- Physical Removal
- Irradiation (UV, IR)
- Reactive Chemical Species (VHP)

**NASA Standard Approaches:**

- Physical Removal
- Vapor Hydrogen Peroxide

## Penetrating

- Addresses interior portions of hardware
- Can include: nested, porous or integrated structures

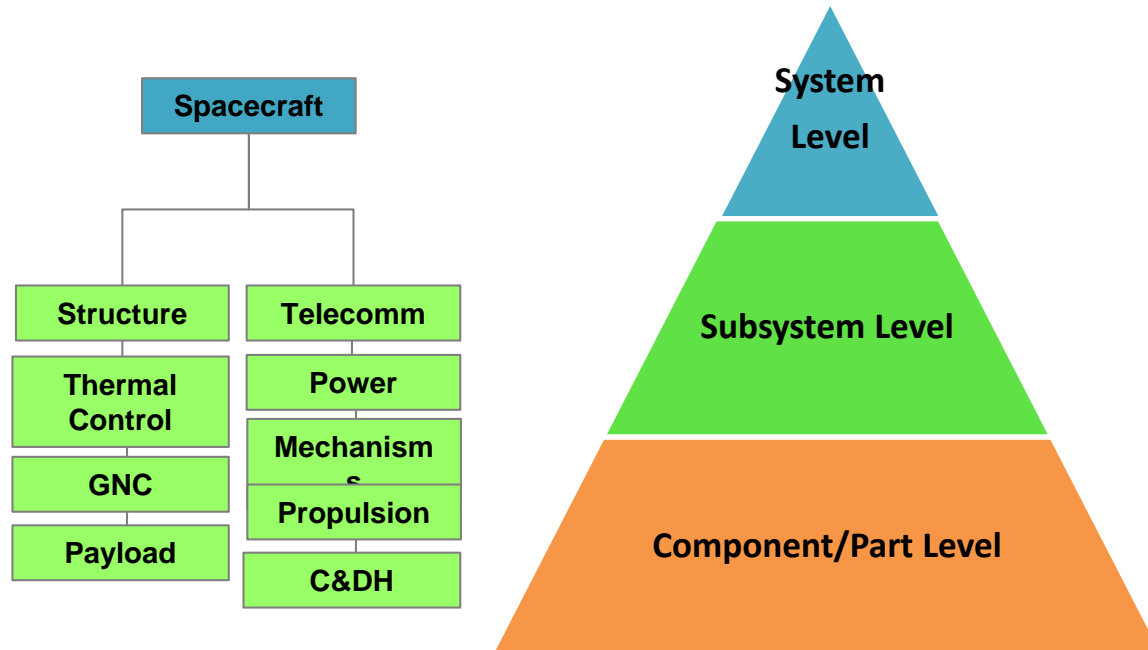
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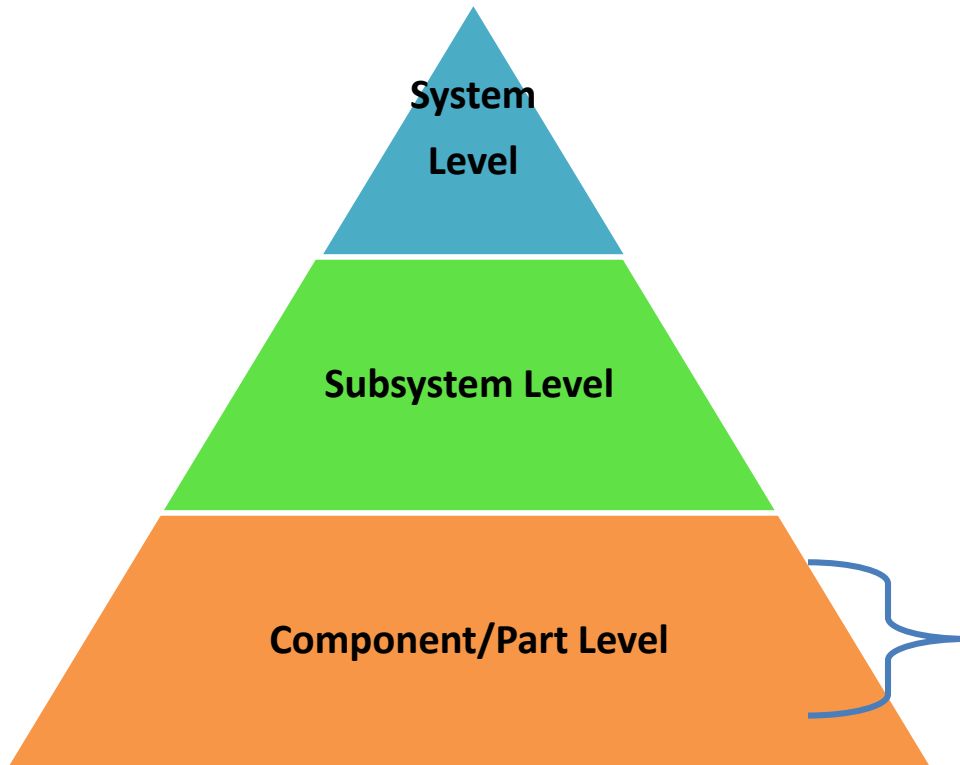
- Dry Heat Microbial Reduction (DHMR)
- Gamma Irradiation
- Gamma + Heat
- Autoclaving

**NASA Standard Approaches:**

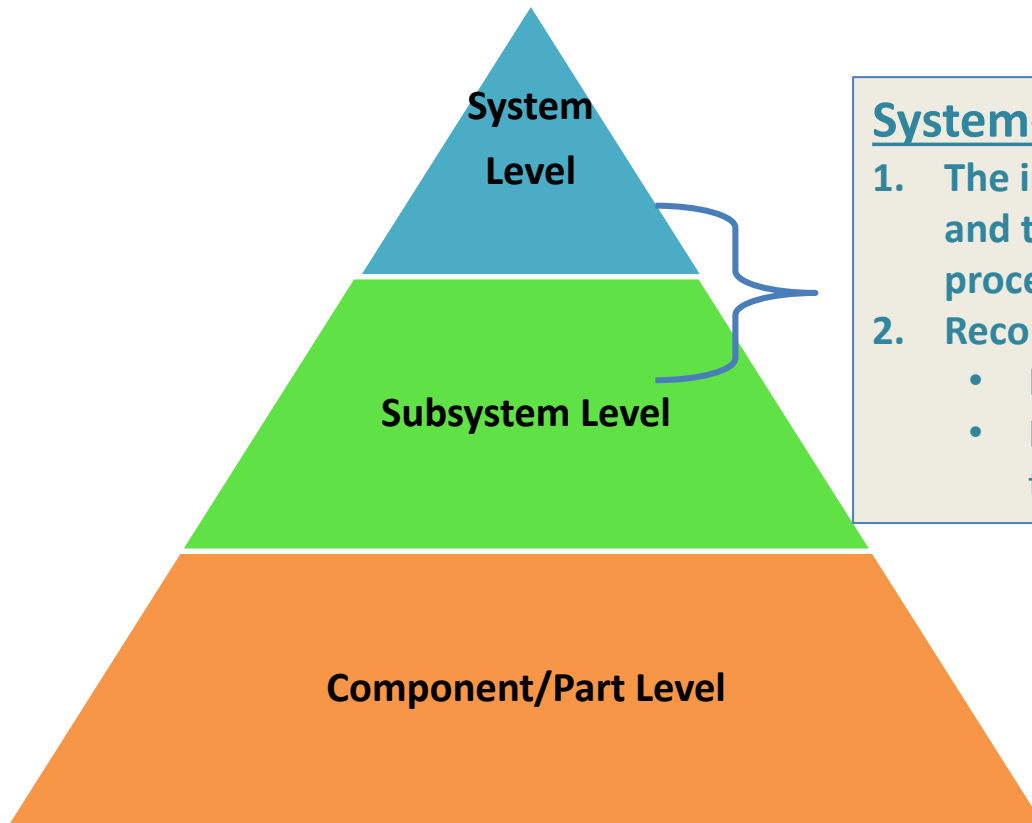
- Dry Heat Microbial Reduction







- **The Stuff:** Materials and Piece Parts compatible with protocols/processes
- **Cleaning the Stuff:** Protocols/Processes to reduce bioburden



### System-Subsystem Level:

1. The interfaces between parts and subsystems and their tolerances to bioburden reduction processes
2. Recontamination prevention:
  - Internal to the system
  - Between the internal and external worlds of the system

# How do we pick a process?

- Materials Compatibility
- Scalability? Piece Parts bioburden reduction? Full-spacecraft?
- Recipe? Is there a NASA standard process? Has NASA accepted someone else's process (ESA)?

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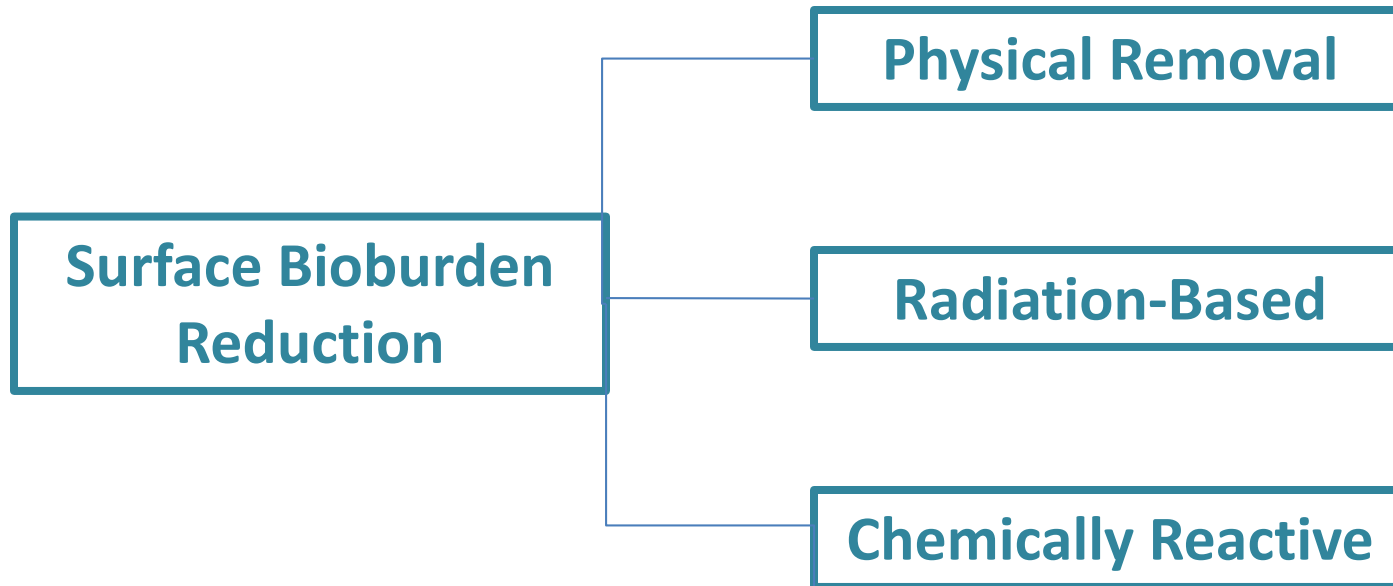
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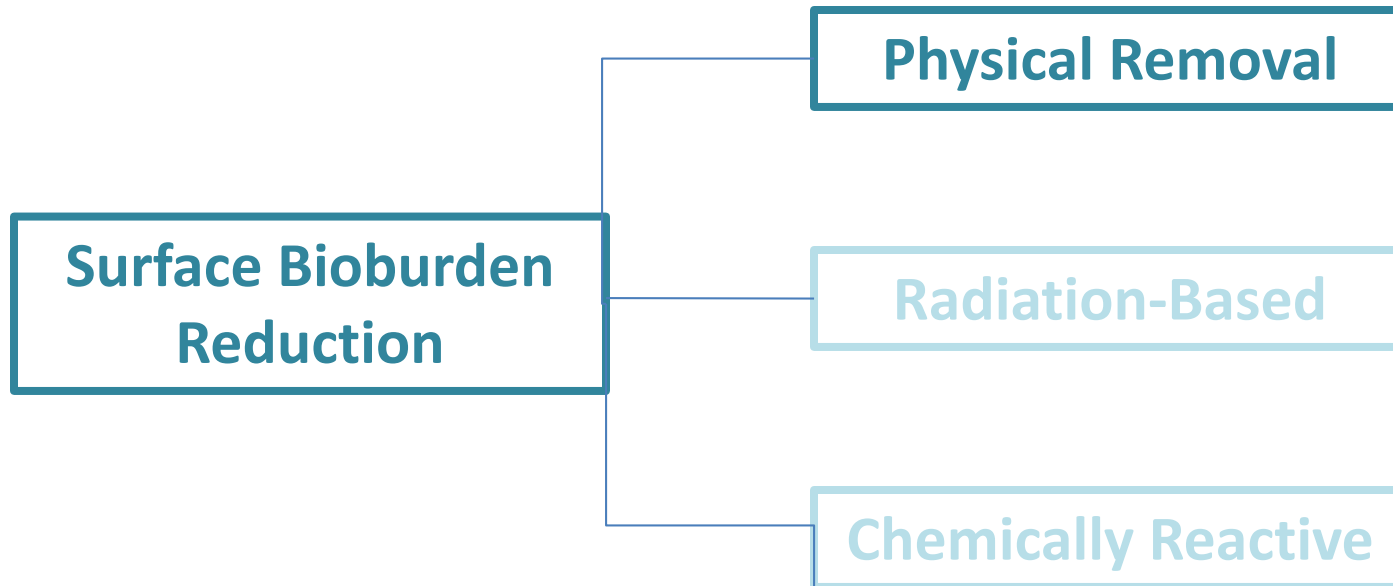
**NASA Standard Approaches:**

- Dry Heat Microbial Reduction

# Brush and Swish: Surface Bioburden Reduction Techniques

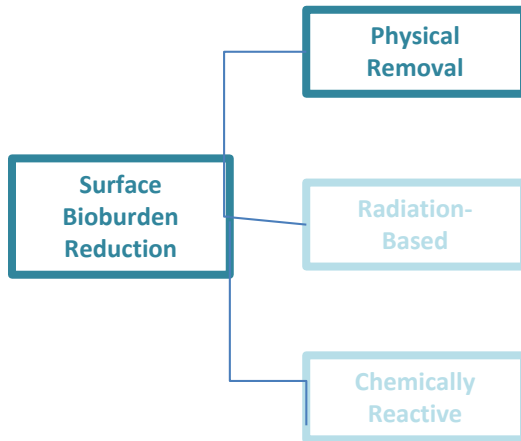


# Brush and Swish: Surface Bioburden Reduction Techniques





# Brush and Swish: Surface Bioburden Reduction Techniques



## Physical Removal

- Solvents
- Foams
- Carbon Dioxide

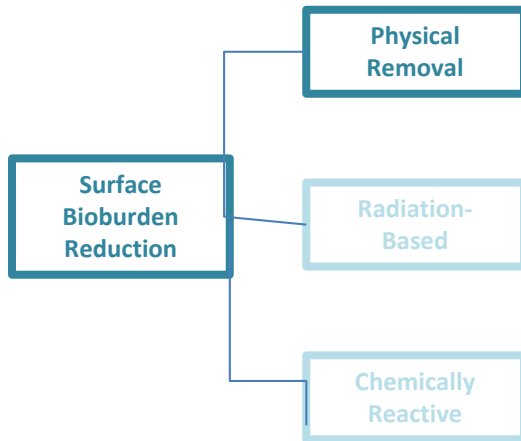
## Solvents

- Often the first line of defense
- Easily accessible
- Work by dissolving or moving surface inhabitants
- Often paired with additional physical removal via wiping and or ultrasound
- Influenced by: hardware surface geometry, surface energy of the solvent, microbial adhesion energy
- A single solvent doesn't always lead to the same log reduction

## Sporicidal Solvents:

- Majority of solvents are not sporicidal. (e.g. IPA, acetone, methanol don't kill spores)
- Sporocidal solvents: glutaraldehyde,  $\text{H}_2\text{O}_2$ , ethylene oxide, chlorine & iodine solvents

# Brush and Swish: Surface Bioburden Reduction Techniques



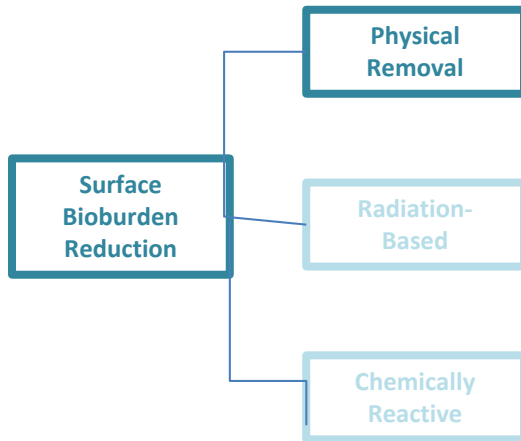
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## Foams

- Commonly used for decontamination of large areas
- Physical nature of foam allows for penetration into various geometries on the size scale of a foam bubble
- Influenced by: the starting biological load on a piece of spaceflight hardware
- Limited studies are available at present, so long-term effects and spore resistance is unknown..
- Scalability? Possibly, due to the physical nature of foams.
- Recipe:? No NASA/ESA standard process.

# Brush and Swish: Surface Bioburden Reduction Techniques



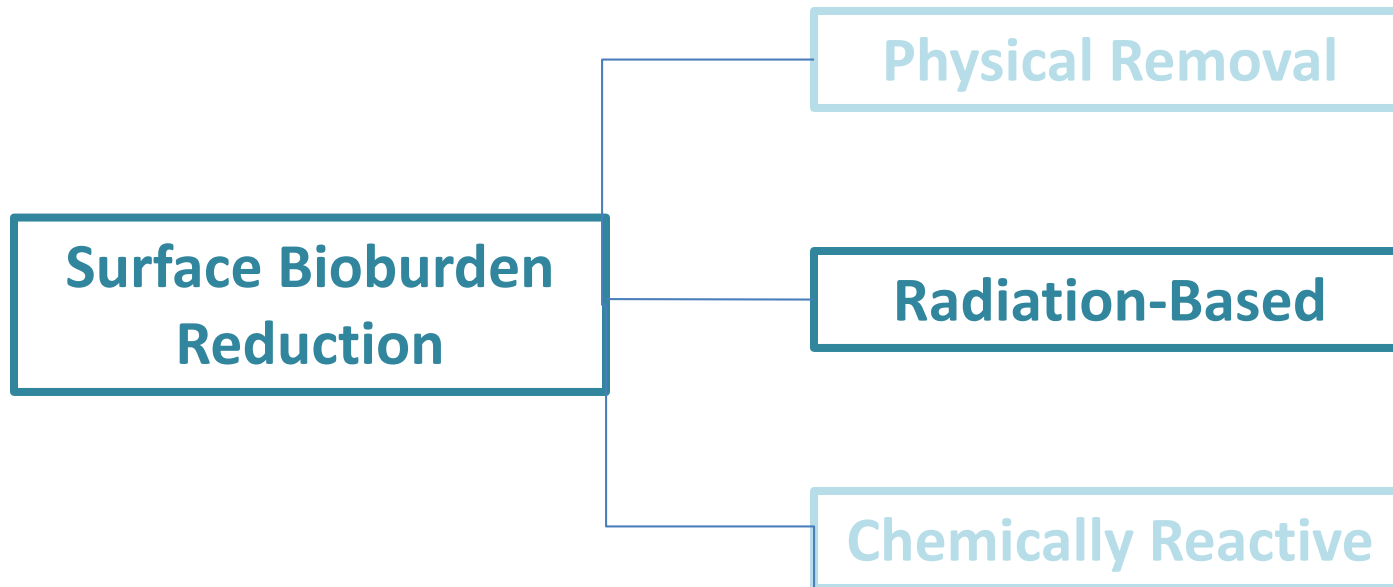
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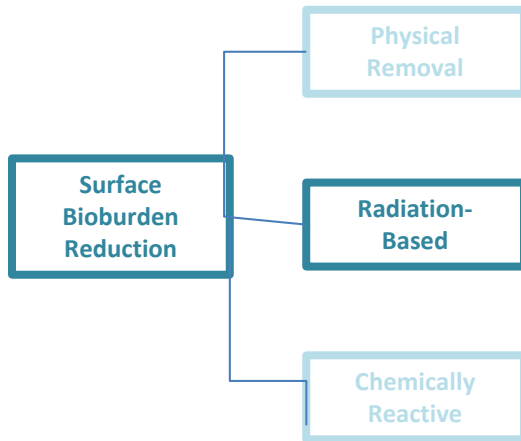
## Carbon Dioxide

- Often employed in the medical and food industry when there are heat- or chemical sensitivity concerns
- Solid CO<sub>2</sub> can be delivered as a particulate so as to bead blast the surface of hardware (CO<sub>2</sub> snow)
- Not effective for spore inactivation, though it is effective for particle removal.
- Majority of work in the literature points to coupling CO<sub>2</sub> with a sporecide or sterile filter
- No NASA/ESA approved processes for use.

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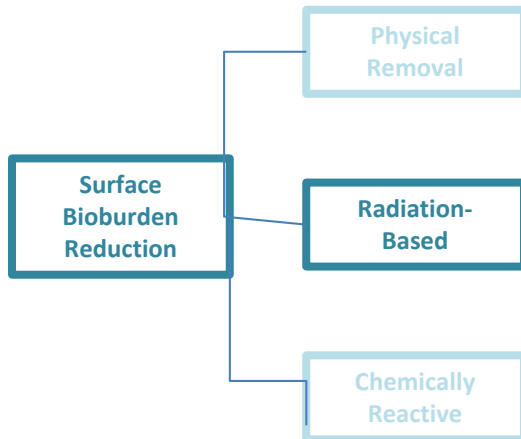
## Radiation-Based

- Ultraviolet
- Infrared

## Ultraviolet (UV)

- Relies on breakdown of microbial DNA at 254 nm. Breakdown results in microbes being unable to reproduce/grow
- Penetration depth is so short that even a layer of spores is sufficient to protect a layer of spores beneath it from harm
- Influenced by: Shadowing, geometry, source-hardware distance, initial level of surface contamination. Cannot be used for interiors or holes
- Can only reach by direct exposure. Scattering length is short
- Spores are able to develop resistance to UV over time.
- Scalability? Possibly
- Recipe:? No NASA/ESA standard process, as results are a mixed bag

# Brush and Swish: Surface Bioburden Reduction Techniques



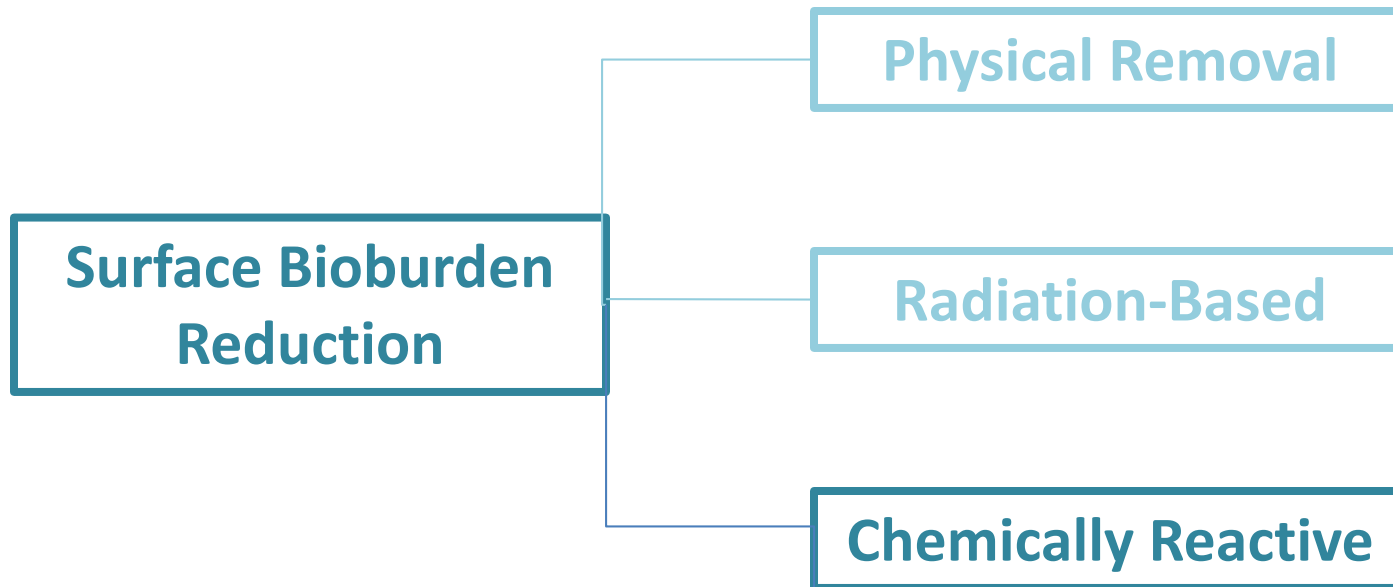
## Radiation-Based

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- Infrared

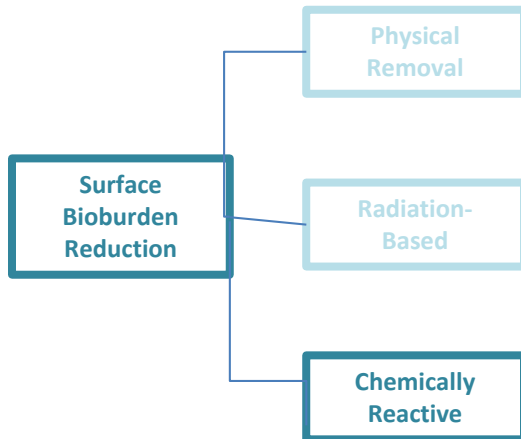
## Infrared (IR)

- Relies on local thermal degradation of the spore coat and internal spore contents
- Influenced by: Shadowing, geometry, source-hardware distance, initial level of surface contamination. Cannot be used for interiors or holes
- Can only reach by direct exposure.
- Mixed results reported in the literature: some reports show increased germination when exposed to IR [J Sawai *et al.* *Heat activation and germination-promotion of Bacillus subtilis spores by infrared radiation*, Int. Biodeter. & Biodg. **63**, 196-200 (2009)]
- Scalability? Possibly
- Recipe:? No NASA/ESA standard process, as results are a mixed bag

# Brush and Swish: Surface Bioburden Reduction Techniques



# Brush and Swish: Surface Bioburden Reduction Techniques



## Chemically Reactive

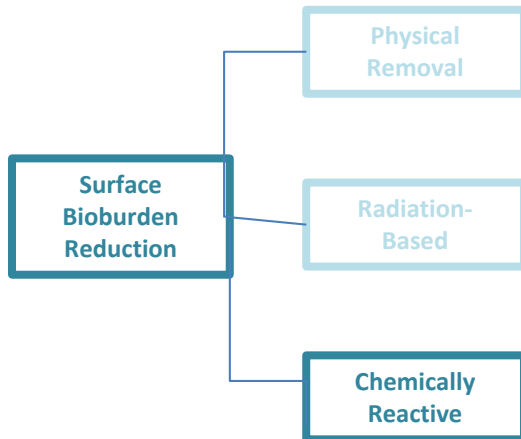
- Plasma
- ~~Ethylene oxide~~
- Nitrogen Dioxide
- Ozone
- Hydrogen Peroxide

## Chemically Reactive Methods

- Relies upon reactive species that are able to disrupt the spore coat and enter into its core to destroy it.
- Often employed in situations where materials would be intolerant of bioburden reduction via techniques that employ high temperatures (DHMR) or humidity (autoclaving)
- Requires some awareness of corrosion susceptibility and etch rates
- Ethylene oxide was popular for NASA use in the 1960s/1970s, but fell out of use due to the Clean Air Act in 1995 (ETO is paired with a Chlorofluorocarbon), so we won't discuss this here.



# Brush and Swish: Surface Bioburden Reduction Techniques



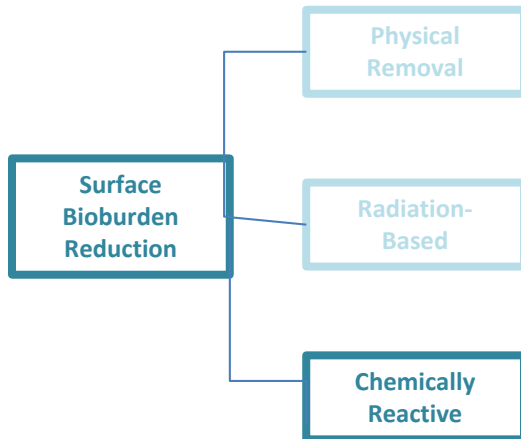
## Chemically Reactive

- Plasma
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## Plasma

- Partially ionized gas composed charged (ons/radicals/electrons) + uncharged species
- Spores are killed via charged species which disrupt the spore coat
- Currently no studies on resistance
- Scalability to large sizes: Potentially, limited by beam size/ rastering capability
- Recipe? No NASA/ESA standard process

# Brush and Swish: Surface Bioburden Reduction Techniques



## Chemically Reactive

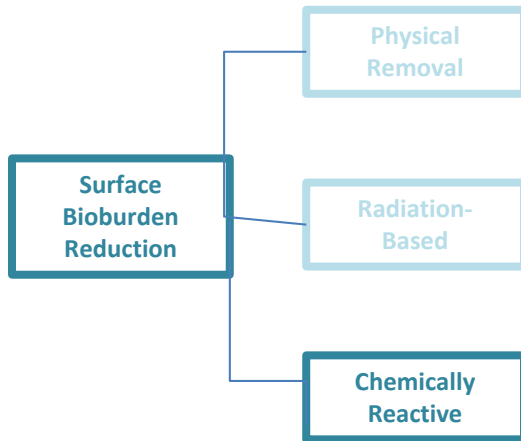
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## Nitrogen Dioxide

- Reactive species of NO<sub>2</sub> gas degrades spore coat
- Can be generated at room temperature
- Cycle times ~ minutes for 6-8 log reduction (*G. stearothermophilus*)
- Resistance: Unknown, limited amount of work done
- Scalability to large sizes: Potentially, limited space in which gas may diffuse
- Recipe? No NASA/ESA standard process

AA Poliakov et al.. Mikrobiologichny Zhurnal, **24** 43-45 (1962).

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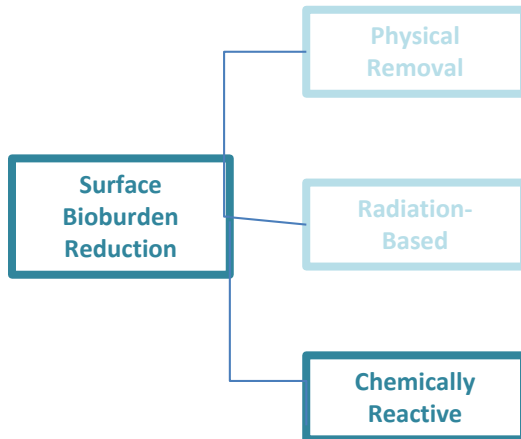
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## Ozone

- Reactive species of oxygen degrades outer coat of spore and inner core.
- Maybe a suitable approach for tool sterilization, as commercial systems are available
- No NASA/ESA standard, but could apply use for tools

# Brush and Swish: Surface Bioburden Reduction Techniques



## Chemically Reactive

- Plasma
- Ethylene oxide
- Nitrogen Dioxide
- Ozone
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## Hydrogen Peroxide

- Vapor Hydrogen Peroxide (VHP) generated by thermal vaporization or pulled by pressure using a carrier gas into a vacuum chamber
- Good materials compatibility for metals and nonmetals
- Scalability? Limited by chamber size
- Recipe? NASA has accepted ESA standard process for use.

# Summary:

## Surface Bioburden Reduction Techniques

### Pro Tips

- **Clean before you reduce!** Without appropriate pre-cleaning, surface bioburden techniques are not as effective as they may seem. (shadowing by organic/particulate matter).
- **Reusing mouthwash or a dirty tooth brush is disgusting at many levels...** Don't recycle solvents or gases reuse. Take care to use ovens/vacuum chambers that have been cleaned or handled with the utmost care for recontamination!
- **Bioburden reduction techniques are not additive:** 1-log reduction by one process+ 1-log reduction by another process does not equal a 2-log reduction!

# Brush and Swish: Surface Bioburden Reduction Techniques

Technique	Log Reduction Range	Possible Spore Resistance?	Residual Dead Bug Bodies
Solvent	NA	Possible	Partially
Foam	4	Unknown	Partially
Ultraviolet	< 2	@low water activity	Yes
Infrared	2-6	Unknown	Yes
Super CO <sub>2</sub>	< 1/None	NA	Partially
NO <sub>2</sub>	4-8	Unknown	Yes
Plasma	2-4	Unknown	Yes
ETO	4	Yes	Partial-none
VHP	4-6	Yes	Partial-none

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### Common Methods:

- Dry Heat Microbial Reduction (DHMR)
- Gamma Irradiation
- Gamma + Heat
- Autoclaving

### NASA Standard Approaches:

- Dry Heat Microbial Reduction



# Deep Cleaning: Penetrating Bioburden Reduction

## Dry Heat Microbial Reduction

- NASA standard process
- T=110-200C for extended durations (e.g 110°C for 50 hours)
- Some organisms are known to be hardy to lower temp/time, leading to spec revision.
- Overlaps exist between DHMR specs and specs for MIL-SPEC 810f-rated parts

## Gamma Radiation

- Inactivation of spores is thought to occur via crosslinking of proteins and generation of radicals when in contact with water
- Room temperature process, though 2.5MRad is what is needed for most spores.

## Gamma + Heat

- Takes advantage of synergy between gamma and heat
- Alternative to DHMR and Gamma, when some parts may be able to withstand some heat and some radiation but not at the levels of standard DHMR and known gamma
- Temperatures = 95-110C and radiation < 150 krad
- 4-7 log reduction occurs in < 24 hours
- May be promising for integrated systems that can withstand standard qualification environments
- Spore resistance is unknown

# Deep Cleaning: Penetrating Bioburden Reduction

Technique	Log Reduction Range	Possible Spore Resistance?	Residual Dead Bodies
DHMR	2-8	Some	Yes
Gamma	2-8	Some	Yes
$\gamma$ + Heat	2-8	Unknown	Yes

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# The Whole Mouth: System-Level Bioburden Reduction

**Say Ah!**

- What we have seen is applicable to piece-parts
- Scaling up to a system-level approach requires work!
- DHMR is the only approach currently approved for system-level bioburden reduction
- The others have promise (e.g. gamma)

Technique		Scalability to Spacecraft System Level
Surface	Foam	Needs development
	Plasma	Needs development
	NO <sub>2</sub>	Needs development
	VHP	Yes, up to specific hardware needs
Penetrating	DHMR	Yes
	Gamma	Yes
	γ + Heat	Needs development



<http://solarsystem.nasa.gov/galleries/viking-in-the-oven>

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# Joints and Interfaces

- Much like how mouthwash has a limited role in getting to the gunk between your teeth, surface bioburden reduction techniques have a limited to zero role in bioburden reduction at joints and interfaces.
- Penetrating approaches such as DHMR have been shown to be effective
- Gamma requires additional work

# Fresh Breath and Clean Teeth

It's possible to take advantage of hardware environmental qualification tests for bioburden reduction purposes: the bonus of fresh breath that you get after brushing your teeth!

## **Parts-Level: Qualification Testing for Europa Environments (Radiation and T):**

*Couple radiation and temperature qualification tests with sterilization qualification testing*

- **EEE Parts Qualification**
- **Structures/Materials Testing**

## **Subsystem/Instrument/System-Level:**

### **Thermal- I&T Campaign Testing:**

*Couple radiation and temperature qualification tests with sterilization qualification testing*

- **TVAC**
- **Contam Bakeouts**



# **Keep Our Solar System Weird**

**[www.planetaryprotection.nasa.gov](http://www.planetaryprotection.nasa.gov)**

# Backup Slides

# An unconscious tribute to Kanye West...*PP Processes*

## Tested

**Heat:** NASA has specifications for time and temperature

*Agency-level testing in the 60s and 70s; project-specific data from the 70s onwards (not public)*

**Vapor Hydrogen Peroxide:** NASA accepts ESA's specs for time-temp-pressure

*ESA has compatibility data*

**CO<sub>2</sub> Snow Cleaning:** ESA has time-pressure specs formally accepted by NASA

*ESA has compatibility data*

**Gamma:** No specifications from NASA or ESA

*Limited compatibility data from NASA from the 60s and 70s*

## Invested

**Plasma Jet Sterilization:** Phase I SBIR, Eagle Harbor Technologies

**CO<sub>2</sub> Snow and Plasma:** Geometric Effects, some materials compat, PPO-funded

**Laser-Induced Plasma Shockwave:** Efficacy and Geometric Effects, PPO-funded

**Vapor Hydrogen Peroxide:** Electronic Packaging Effects, PPO-funded

## Unexplored, but interested...

**Electron beam**

**Ozone**

**Nitrogen Dioxide**

**X-ray:** Very limited testing

## Retro, chango and maybe a go? Or maybe a no?

**Terminal Sterilization by use of thermite:** explosive sterilization at EoM: Goddard

**Iodine Marking of spacecraft:** Messy! SETI



# OK, Kanye, but we're not alone...

USDA  
FDA  
CDC  
NIH  
HHS  
DHS

**Heat:** Majority are steam heat implementations (vs. dry heat)  
**CO<sub>2</sub> Snow Cleaning:** Not used by others  
**Gamma:** A lot of research by CDC, but no specs: Cost-prohibitive for them (but maybe not for us)  
**References for the curious:**  
*CFR Chapter 21 – Part 110 (USDA, Food)*  
*CDC Guidelines for Disinfection/Sterilization*  
[https://www.cdc.gov/hicpac/pdf/guidelines/Disinfection\\_Nov\\_2008.pdf](https://www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.pdf)

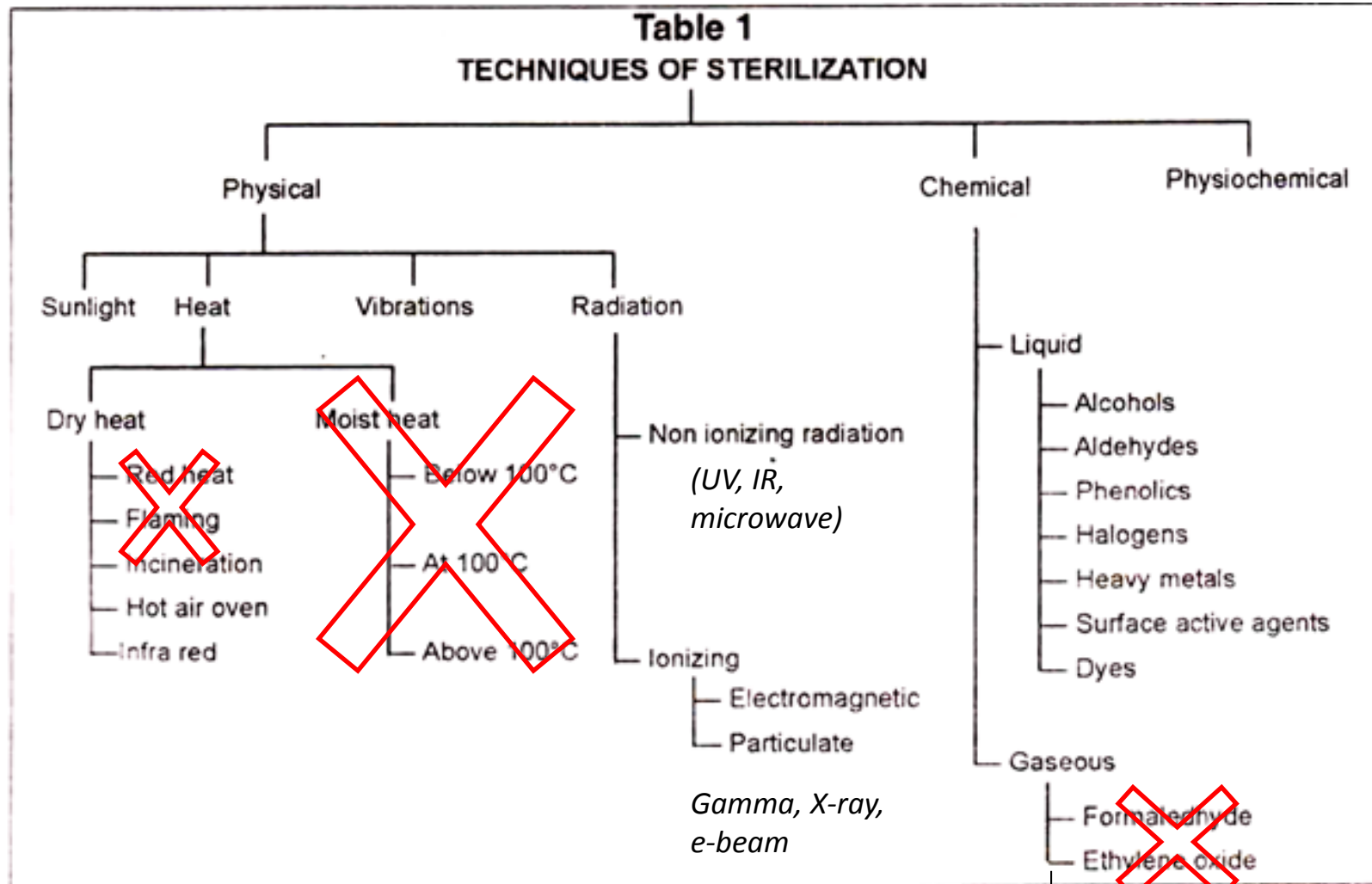
**Military**

**MIL SPEC 810 Rev G, Method 501.5-** Procedure I nonoperational, high temperature testing and Procedure III – operational at room temperature, after high temperature testing  
    Temperature Range: -40° to +125°C, nonoperational  
    Duration  
    Equivalent to DHMR  
**MIL 5090 : High Temperature Adhesives**  
**MIL : High Temperature Structures for Airframes**  
**Vapor Hydrogen Peroxide :** Use for anthrax, sans organic contam.

**Automotive Industry**

**Automotive Electronics Qualified Parts AEC-Q100, -Q101, -Q200:**  
    Temperature Range: -40°C to +125°C, operational (!)  
    Microcircuits, Discrete Semiconductors and Passive Components  
    AEC-Q100 is a set of reliability stress tests to qualify integrated circuits for automotive applications

# What others do that NASA won't...



Vapor Hydrogen Peroxide

**= Tested and not used by NASA due to compatibility/lifetime issues**